BRIEF COMMUNICATION

Maternal Genetic Effects on Neonatal Susceptibility to Oxidative Damage From Environmental Tobacco Smoke

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Molecular epidemiologic studies (1,2) have demonstrated an association between environmental pollutants and *in utero* developmental damage. Among the environmental pollutants to which developing fetuses are reported to be vulnerable is environmental tobacco smoke (ETS) (3). ETS is a complex mixture of volatiles and particulate matter comprising numerous compounds, including polycyclic aromatic hydrocarbons (PAHs), which cause oxidative damage (4). Maternal ETS exposure has been found to be an important risk factor for reduced birth weight, small-for-gestational age, and premature delivery (5–8). Recently, biologic monitoring methods for the measurement of PAH exposure and the resulting oxidative injury have been used to evaluate the effects of environmental pollutant exposure (9,10). Sensitive biologic markers allow a more accurate quantification of PAH exposure levels and early biologic effects than external exposure levels as estimated by environmental monitoring and the use of standard health outcomes. Biomarker levels in response to a given PAH exposure vary between individuals, and genetic differences may be a cause of this variability (11,12). Genetic polymorphisms have been detected in a variety of enzymes involved in the activation and detoxification of PAHs (12–17).

Our goal was to determine whether toxic metabolites of ETS cause neonatal oxidative damage and, if so, whether maternal genetic polymorphisms modulate the damage.

To examine these questions, we conducted a study among mothers at delivery and neonates in Inchon, Korea. The study included 81 mothers who had not smoked cigarettes during pregnancy and 20 of their newborns. All eligible pregnant women hospitalized for delivery during the 6 months of enrollment (January 1, 1999, to June 13, 1999) were invited to participate in this study, and 105 agreed. The eligibility criteria included being local residents in stable medical condition, with no evidence of pregnancy toxemia, hypertension, diabetes mellitus, thyroid disease, bronchial asthma, active hepatitis, chronic renal failure, or heart failure. Maternal blood and urine samples were collected at admission, and 24 women were excluded from the final analysis because their blood and urine samples were inadequate for laboratory analysis. Other than height, the general characteristics of the excluded women were not different from those of the final study participants. Neonatal urine samples were collected at a neonatal care unit within 24 hours of birth. However, because it is difficult to collect urine samples from neonates, samples were available for only 26 newborns, six of whom were excluded from the analysis because their maternal data were not adequate. The sex ratio and birth weight of the study neonates were similar to those of the nonparticipating neonates. The study was approved by the Inha University Hospital Institutional Review Board, and written informed consent was obtained from all maternal participants.

Questionnaires were administered to the mothers at the time of blood sampling to obtain information on ETS exposure, residential and employment history, alcohol consumption, and diet. The assessment of smoking status was based on questionnaire data, and the extent of exposure to ETS was based on radioimmunoassay analysis of urinary cotinine. For maternal PAH exposure biomarkers, we measured urinary 1-hydroxypyrene and 2-naphthol levels by using high-performance liquid chromatography (HPLC) with a fluorescence detector (9). For neonatal oxidative injury biomarkers, we measured the levels of urinary 8-hydroxy-deoxyguanosine (8-OHdG) by using a competitive enzyme-linked immunosorbent assay (10), and we determined the levels of malondialdehyde (MDA) by HPLC of the adduct obtained with thiobarbituric acid (18).

We determined the maternal geno- types for four enzymes that are involved in the activation (cytochrome P450 [CYP] 1A1 and CYP2E1) and detoxification (glutathione S-transferase [GST] M1 and GSTT1) of PAHs. We used polymerase chain reaction (PCR) analysis to examine two different polymorphisms in CYP1A1, *MspI* (T6235C) in the 3' flanking region (19) and Ile/Val (A4889G) in exon 7 (20), and one polymorphism in CYP2E1, *PstI* (G-1259C) in the 5' flanking region (21). For GSTM1 and GSTT1, we used PCR to examine gene deletion and simultaneously amplified a 268-base-pair fragment of the β-globin gene as an internal positive control (22,23). The homozygous and heterozygous allelic variants of CYP1A1 and CYP2E1 were combined in the analysis because of the small numbers of the homozygous allelic variants (data not shown).

Urinary concentrations of biomarkers were adjusted to the urinary concentration of creatinine to control for variations in urine flow, and log-transformed concentrations were used to stabilize the variance. Geometric means and 95% confidence intervals were presented as measures of the data distribution. Associations between maternal exposure to ETS and oxidative damage in fetuses were evaluated initially by Student's *t* test and subsequently by multivariate analysis. Regression models on fetal oxidative injury included maternal ETS exposure, maternal genotypes of enzymes involved in PAH metabolism, maternal age, neonatal sex, birth weight, pregnancy duration, and delivery type (vaginal delivery or cesarean section).

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Least-square means were used to compare the concentrations of oxidative biomarkers among neonatal subgroups. All statistical tests were two-sided.

Table 1 shows the urinary concentrations of maternal exposure and neonatal oxidative injury biomarkers among the study subjects. Neonatal urinary 8-OHdG concentrations were statistically significantly associated with maternal exposure to ETS ($P = .047$). In the analysis of the effect of genotype, neonatal urinary 8-OHdG levels were also statistically significantly elevated with maternal GSTM1 null genotype ($P = .007$) (data not shown). These statistically significant relationships were maintained in various regression models adjusted for other potential confounders. Maternal genotypes of CYP1A1, CYP2E1, and GSTT1 did not have statistically significant effects on neonatal urinary 8-OHdG concentrations (data not shown). Neonatal urinary MDA excretions were not affected statistically significantly by maternal genotypes or exposure to ETS. When neonatal urinary 8-OHdG concentrations were grouped according to maternal ETS exposure status and GSTM1 genotype, there were statistically significant differences among some of the subgroups (Fig. 1). The concentration was highest in newborns whose mothers were exposed to ETS and carry the GSTM1 null genotype and was lowest in mothers not exposed to ETS and carrying the wild-type GSTM1 allele. These results indicate that oxidative damage in newborns is affected by both exposure to ETS and maternal GSTM1 genotype.

Although maternal exposure levels to ETS were relatively low and the neonatal sample size was small in this study, our findings show that neonatal urinary 8-OHdG concentrations were statistically significantly associated with maternal exposure to ETS, after controlling for other covariates. Substantial household exposure sources of PAHs, in addition to cigarette smoking, include ambient particulates, smoke from cooking, and diet. However, we found no association between other sources of maternal PAH exposure and neonatal oxidative injury biomarkers in urine (data not shown).

Individuals with the GSTM1 null genotype are expected to detoxify PAHs less efficiently than individuals with functional GSTM1, thus increasing concentrations of toxic metabolites in the body (14,24,25). Our results show that the concentrations of neonatal urinary 8-OHdG are statistically significantly higher when mothers have the GSTM1 null genotype than when mothers have the GSTM1 wild-type genotype (Fig. 1). These results suggest that the GSTM1 genotype affects the transfer of toxic chemicals from mother to fetus.

Our findings strongly show the need for smoking cessation among household members during pregnancy. Because neonatal urinary 8-OHdG levels were also statistically significantly elevated with maternal GSTM1 null genotype in the presence or absence of ETS exposure, strategies to prevent oxidative damage to fetuses should require identification of individuals susceptible to environmental toxicant exposure.
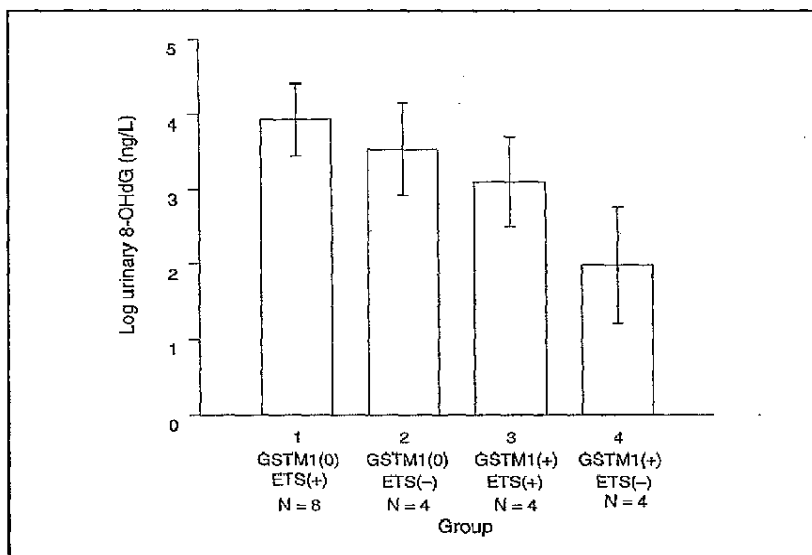

Fig. 1. Concentrations of neonatal urinary 8-hydroxy-deoxyguanosine (8-OHdG) among subgroups defined by maternal glutathione S-transferase M1 (GSTM1) genotype and environmental tobacco smoke (ETS) exposure. Error bars represent 95% confidence intervals. P values for pairwise comparisons when least-square means were compared by a general linear model after controlling for maternal age, neonatal sex, birth weight, pregnancy duration, and delivery type are as follows: group 1 versus group 2, $P = .31$; group 1 versus group 3, $P = .049$; group 1 versus group 4, $P = .002$; group 2 versus group 3, $P = .29$; group 2 versus group 4, $P = .007$; and group 3 versus group 4, $P = .023$.

Table 1. Urinary concentrations of biomarkers of maternal exposure to ETS and neonatal oxidative injury*

Biomarker	GM (95% CI)	
	ETS-nonexposed mothers	ETS-exposed mothers
Maternal exposure biomarker		
No. of maternal subjects	37	44
Cotinine, μg/g creatinine	8.61 (4.84 to 9.51)	12.11 (7.34 to 19.98)
1-OHP, μg/g creatinine	0.13 (0.10 to 0.14)	0.16 (0.12 to 0.21)
2-naphthol, μg/g creatinine	3.34 (2.27 to 3.56)	4.61 (3.37 to 6.30)
Neonatal oxidative injury biomarker		
No. of neonatal subjects	8	12
8-OHdG, μg/L†	0.93 (0.24 to 1.50)	4.03 (2.13 to 7.61)
MDA, mmol/L	0.69 (0.42 to 0.83)	0.76 (0.56 to 1.05)

*ETS = environmental tobacco smoke; GM = geometric mean; CI = confidence interval; 1-OHP = 1-hydroxypyrene; 8-OHdG = 8-hydroxy-deoxyguanosine; MDA = malondialdehyde.
†Statistically significant difference between ETS-nonexposed and ETS-exposed groups with the use of Student's t test ($P = .047$).

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